PATENT COOPERATION TREATY

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		Date of mailing (day/month/year) see	form PCT/ISA/210 (second sheet)
pplicant's or agent's file reference ee form PCT/ISA/220		FOR FURTHER A See paragraph 2 below	w
nternational application No.	International filing date (da 07.04.2005	y/month/year)	Priority date (day/month/year) 07.04.2004
nternational Patent Classification (IPC) or C12N15/75, C12N1/21	Legistrational classification are	nd IPC	
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□ Box No. IV Lack of unity of Reasoned state applicability; of Box No. VI Certain docurting Box No. VII Certain defection Box No. VIII Certain observations and the control of the contro	ment of opinion with regar of invention tement under Rule 43 <i>bis</i> . itations and explanations nents cited ts in the international appl	rd to novelty, inventing (a)(i) with regard to supporting such sta	ve step and industrial applicability o novelty, inventive step or industrial tement
written opinion of the Internatio the applicant chooses an Autho International Bureau under Rul will not be so considered.	nal Preliminary Examining ority other than this one to e 66.1 <i>bis</i> (b) that written o	be the IPEA and the pinlons of this Intern	,
			PPEA, the applicant is Invited to nents, before the expiration of three n of 22 months from the priority date,
For further options, see Form I	PCT/ISA/220.	_	·
3. For further details, see notes to	Form PGT/ISA/220.	·	
Name and mailing address of the ISA:		Authorized Officer	When to



European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016

Montero Lopez, B

Telephone No. +31 70 340-3739



WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/DK2005/000236

	Вох	No	o. I Basis of the opinion			
1.	With	re lanç	gard to the language , this opinion has been established on the basis of the international application in guage in which it was filed, unless otherwise indicated under this item.			
		lan	is opinion has been established on the basis of a translation from the original language into the following guage , which is the language of a translation furnished for the purposes of international search or roles 12.3 and 23.1(b)).			
2.	With	ı re ess	gard to any nucleotide and/or amino acid sequence disclosed in the international application and ary to the claimed invention, this opinion has been established on the basis of:			
	a. ty	pe	of material:			
	2	3	a sequence listing			
	[3	table(s) related to the sequence listing			
	b. format of material:					
	Ē	⊠	in written format			
	Ē	Ճ	in computer readable form			
	c. ti	me	of filling/furnishing:			
		⊠	contained in the international application as filed.			
	Ē	Ø	filed together with the international application in computer readable form.			
	[J	furnished subsequently to this Authority for the purposes of search.			
3.		ha co	addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto s been filed or furnished, the required statements that the information in the subsequent or additional pies is identical to that in the application as filed or does not go beyond the application as filed, as propriate, were furnished.			
4.	Add	litio	nal comments:			
_	Во	N	o. VIII Certain observations on the international application			

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

PCT/DK2005/000236

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

- D1: KYOKO KANAMARU ET AL.: "Overexpression of the PepF oligopeptidase inhibits sporulation initiation in Bacillus subtilis" JOURNAL OF BACTERIOLOGY, vol. 184, no. 1, January 2002 (2002-01), pages 43-50, XP002334436
- 1. The present application relates to the production of heterologous proteins in Bacillus cells which have been mutated to reduce the expression-levels of the YusZ or YusX genes.
- 2. Document D1 discloses that B. subtilis YusX gene encode an oligoendopeptidase and that YusX and YusY may be a single ORF. The function of the YusXY protein appears to be unknown. There is no hint in the state of the art to indicate the skilled person that reduced expression of YusX or YusZ in Bacillus would improve secretion of heterologous proteins. Claims 1-57 therefore are novel and inventive and comply with the requirements of Articles 33(2) and (3) PCT.

Re Item VIII

Certain observations on the international application

- 1. Claims 1, 5, 6, 20, 25, 39 and 44 include the sequence of the Yus protein or polynucleotide between parenthesis. This renders the sequence an optional feature which does not have any limiting effect in the scope of the claims. The deletion of the parenthesis and specification of the sequence would improve the clarity of the claims as requested according to Article 6 PCT.
- 2. The relative term "homologue" used in claims 1, 5-8, 10-13, 20, 24-27, 29-32, 39, 43-46, 48, 50 and 51 has no well-recognised meaning and leaves the reader in doubt as to the

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.

PCT/DK2005/000236

meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT). It is unclear what sort of homology (functional, structural) is meant and what the degree of homology should be.

- 3. The relative term "polypeptide of interest" used in claims 1, 20 and 39 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).
- 4. The feature of claims 6-11, 25-30 and 44-49 of a mutation in the YusY gene is not supported by the examples. The examples show only the expected effect for YusX and YusZ deletion mutants. Claims 6-11, 25-30 and 44-49 are therefore not supported by the description as required by Article 6 PCT.
- 5. Claims 19, 38 and 57 include an optional feature (preferably derived from the Cryllla promoter) which does not have any limiting effect in the scope of the claims. The deletion of this feature would improve the clarity of the claims as requested according to Article 6 PCT.

PATENT COOPERATION TREATY

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	ational application N/DK2005/000236		International filing date 07.04.2005	(day/month/year)	Priority date (day/month/year) 07.04.2004
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European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016 Montero Lopez, B

Telephone No. +31 70 340-3739



WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/DK2005/000236

Box No. 1 Basis of the opinion 1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item. This opinion has been established on the basis of a translation from the original language into the following language, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)). 2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of: a. type of material: a sequence listing b format of material: in computer readable form c. time of filing/furnishing: contained in the international application as filed. filed together with the international application in computer readable form. furnished subsequently to this Authority for the purposes of search. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.						
the language in which it was filed, unless otherwise indicated under this item. This opinion has been established on the basis of a translation from the original language into the following language which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)). With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of: a. type of material: a. a sequence listing table(s) related to the sequence listing b. format of material: in written format c. time of filing/furnishing: contained in the international application as filed. filed together with the international application in computer readable form. furnished subsequently to this Authority for the purposes of search. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.	_	Вох	No. I	Basis of the opinion		
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Box No. VIII Certain observations on the international application		Box	No. V	/III Certain observations on the international application		

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Form PCT/ISA/237 (January 2004)

PCT/DK2005/000236

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

- D1: KYOKO KANAMARU ET AL.: "Overexpression of the PepF oligopeptidase inhibits sporulation initiation in Bacillus subtilis" JOURNAL OF BACTERIOLOGY, vol. 184, no. 1, January 2002 (2002-01), pages 43-50, XP002334436
- 1. The present application relates to the production of heterologous proteins in Bacillus cells which have been mutated to reduce the expression-levels of the YusZ or YusX genes.
- 2. Document D1 discloses that B. subtilis YusX gene encode an oligoendopeptidase and that YusX and YusY may be a single ORF. The function of the YusXY protein appears to be unknown. There is no hint in the state of the art to indicate the skilled person that reduced expression of YusX or YusZ in Bacillus would improve secretion of heterologous proteins. Claims 1-57 therefore are novel and inventive and comply with the requirements of Articles 33(2) and (3) PCT.

Re Item VIII

Certain observations on the international application

- 1. Claims 1, 5, 6, 20, 25, 39 and 44 include the sequence of the Yus protein or polynucleotide between parenthesis. This renders the sequence an optional feature which does not have any limiting effect in the scope of the claims. The deletion of the parenthesis and specification of the sequence would improve the clarity of the claims as requested according to Article 6 PCT.
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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.

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